

APPROVED	C.G. FIG.	
BY	CLASS	SUBCLASS
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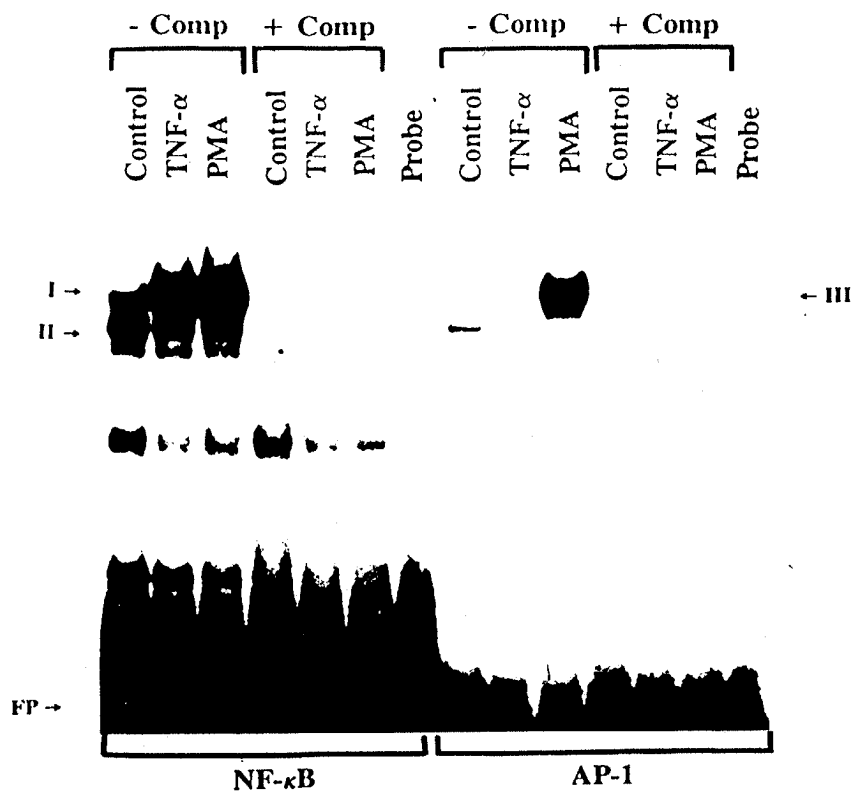


Fig. 1

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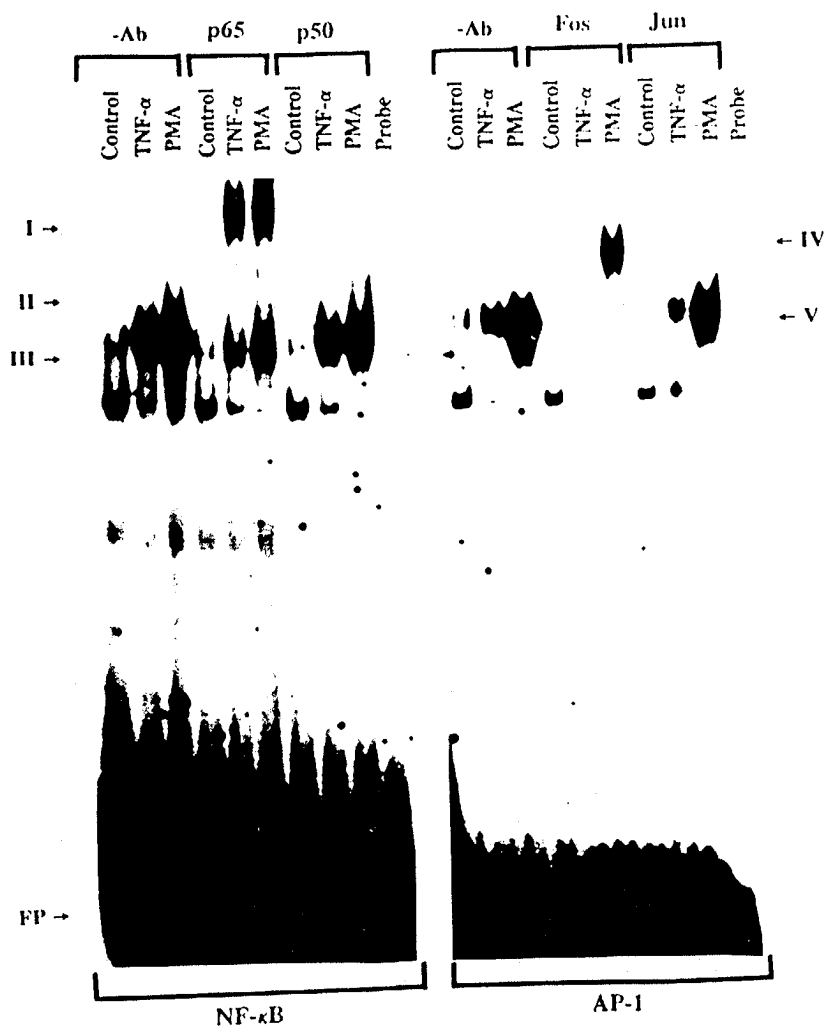


Fig. 2

APPROVED	D.G. FIG.	
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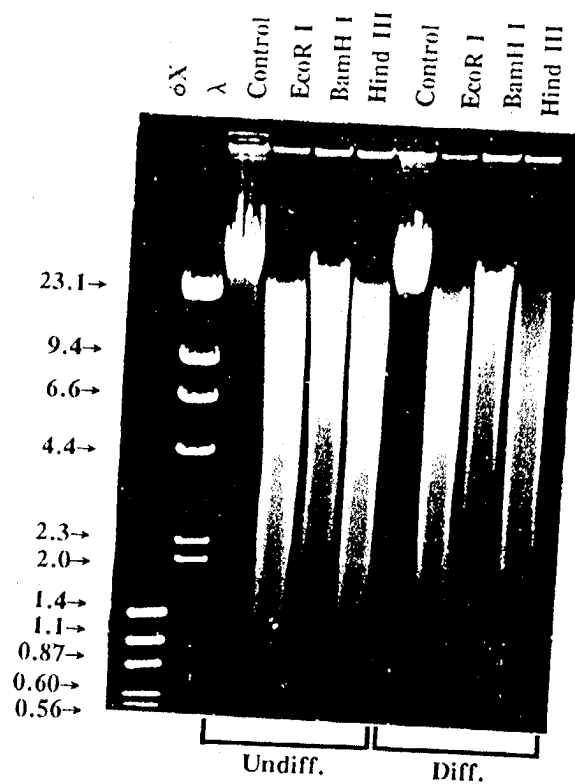


Fig. 3

APPROVED	O.G. FIG.	
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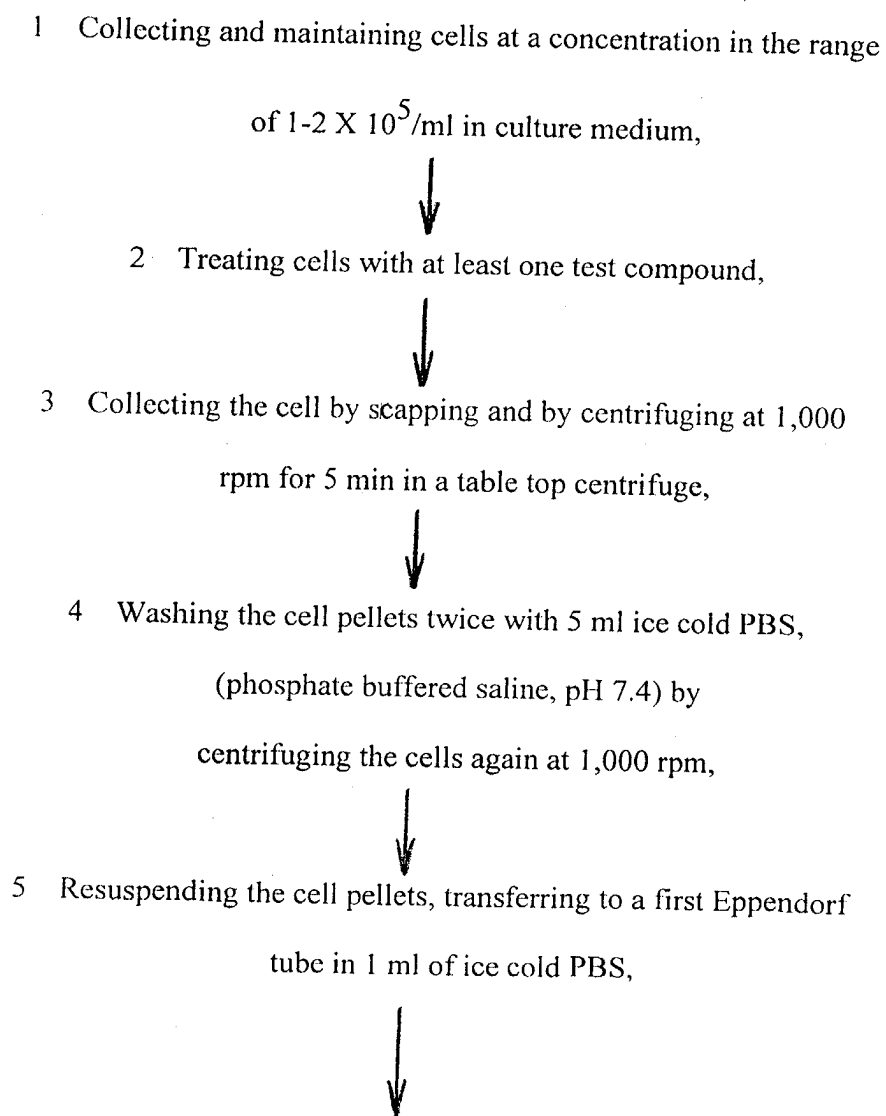


Fig. 4 A

APPROVED	D.B. FIG.	
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- 6 Centrifuging at 2,000 rpm for 5 min at 4 °C removing the PBS processing the cell pellets following according to the protein and DNA isolation steps comprising,



- 7 Preparing Buffer A: (cell lysis buffer), [20 mM Hepes, pH 7.9, 10 mM NaCl, 3 mM MgCl₂, 0.1% NP-40, 10% glycerol, 0.2 mM EDTA, 1 mM dithiothreitol (DTT), 0.4 mM phenylmethylsulfonyl fluoride (PMSF), antipain (1 µg/ml), leupeptin (1 µg/ml)],



- 8 Preparing Buffer B: (extraction buffer without salt), [20 mM Hepes, pH 7.9, 0.2 mM EDTA, 20% glycerol, 1 mM DTT, 0.4 mM PMSF, antipain (1 µg/ml), leupeptin (1 µg/ml)],



- 9 Preparing Buffer C: (extraction buffer with salt), [20 mM Hepes, pH 7.9, 400 mM NaCl, 0.2 mM EDTA, 20% glycerol, 1 mM DTT, 0.4 mM PMSF, antipain (1 µg/ml), leupeptin (1 µg/ml)],



Fig. 4 B

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APPROVED	C.G. FIG.	
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10 Preparing Buffer D: (cytoplasmic extraction clarification buffer), [20 mM Hepes, pH 7.9, 400 mM NaCl, 0.2 mM EDTA, 40% glycerol, 1 mM DTT, 0.4 mM PMSF, antipain (1 $\mu\text{g/ml}$), leupeptin (1 $\mu\text{g/ml}$)],



11 Performing Simultaneous isolation of protein and DNA comprising the steps of,



12 Resuspending the cell pellets in 100-125 μl (2 pellet vol) of Buffer A,



13 Maintaining the resuspended cell pellets on ice for 10-15 min with occasional tapping,



14 Pelleting the nuclei by centrifuging at 2,000 rpm for 5 min at 4°C,



15 Removing the cytoplasmic supernatant fraction to a second Eppendorf tube,



Fig. 4 C

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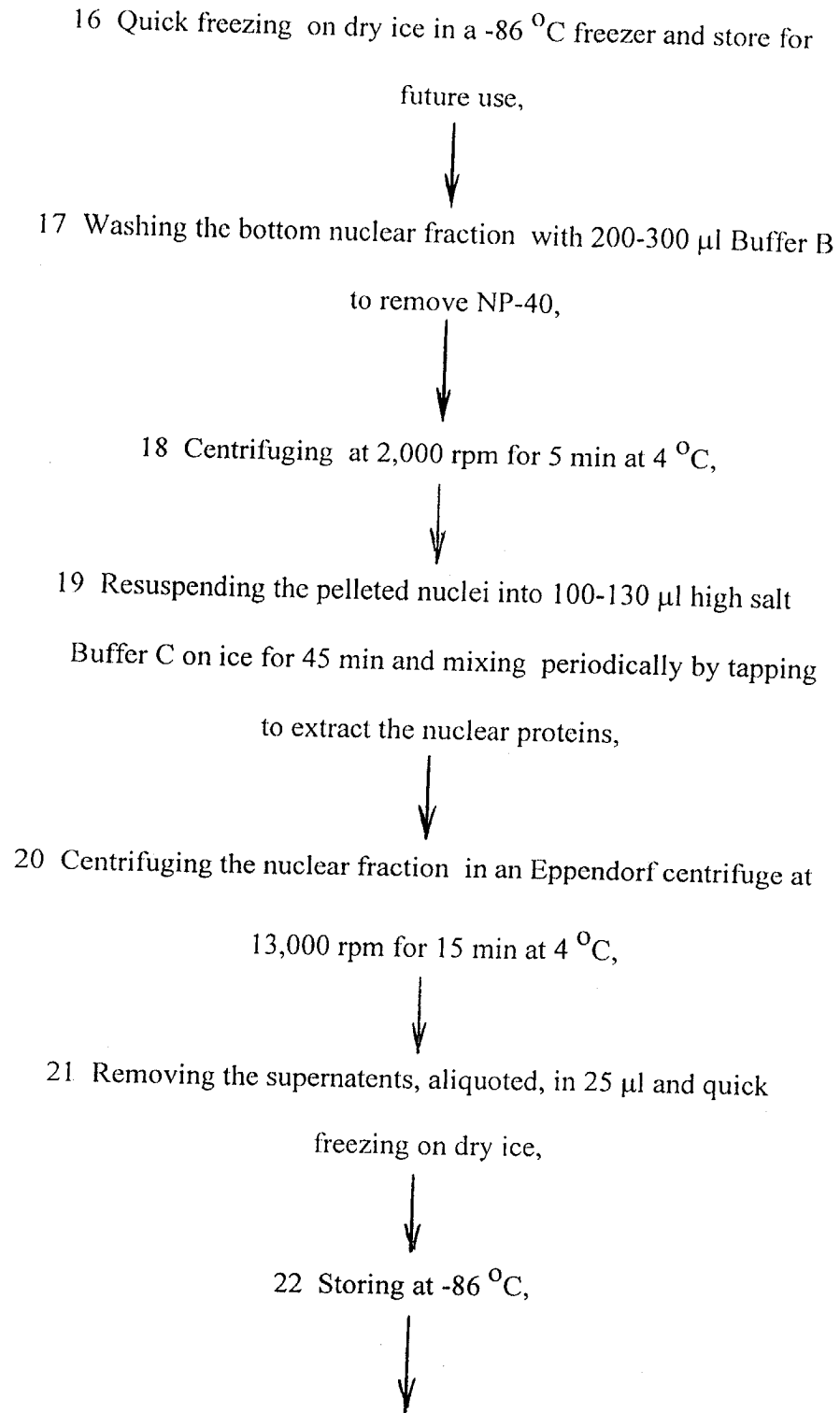


Fig. 4 D

APPROVED	B.G. FIG.	
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23 Quick freezing the remaining pellet containing nucleic acids

and other debris,



24 Storing at -86°C ,



25 Clarifying the cytoplasmic fraction by adding 1/3 vol of
Buffer D to this fraction for 30 min at 4°C to equilibrate the

cytoplasmic proteins with NaCl,



26 Centrifuging at 13,000 rpm for 15 min.,



27 Removing and quick freezing the supernatants and storing at -
 86°C ,



28 Performing DNA extraction and analysis comprising the steps
of,



29 Thawing frozen cell pellets from on ice for 10 min,



30 Adding 100 μl of Buffer [0.1 % SDS, 10 mM Tris-HCl, pH 7.9,
10 mM EDTA, 10 mM NaCl] for 15 min,



Fig. 4 E

004050-2056960

APPROVED	D.G. FIG.	
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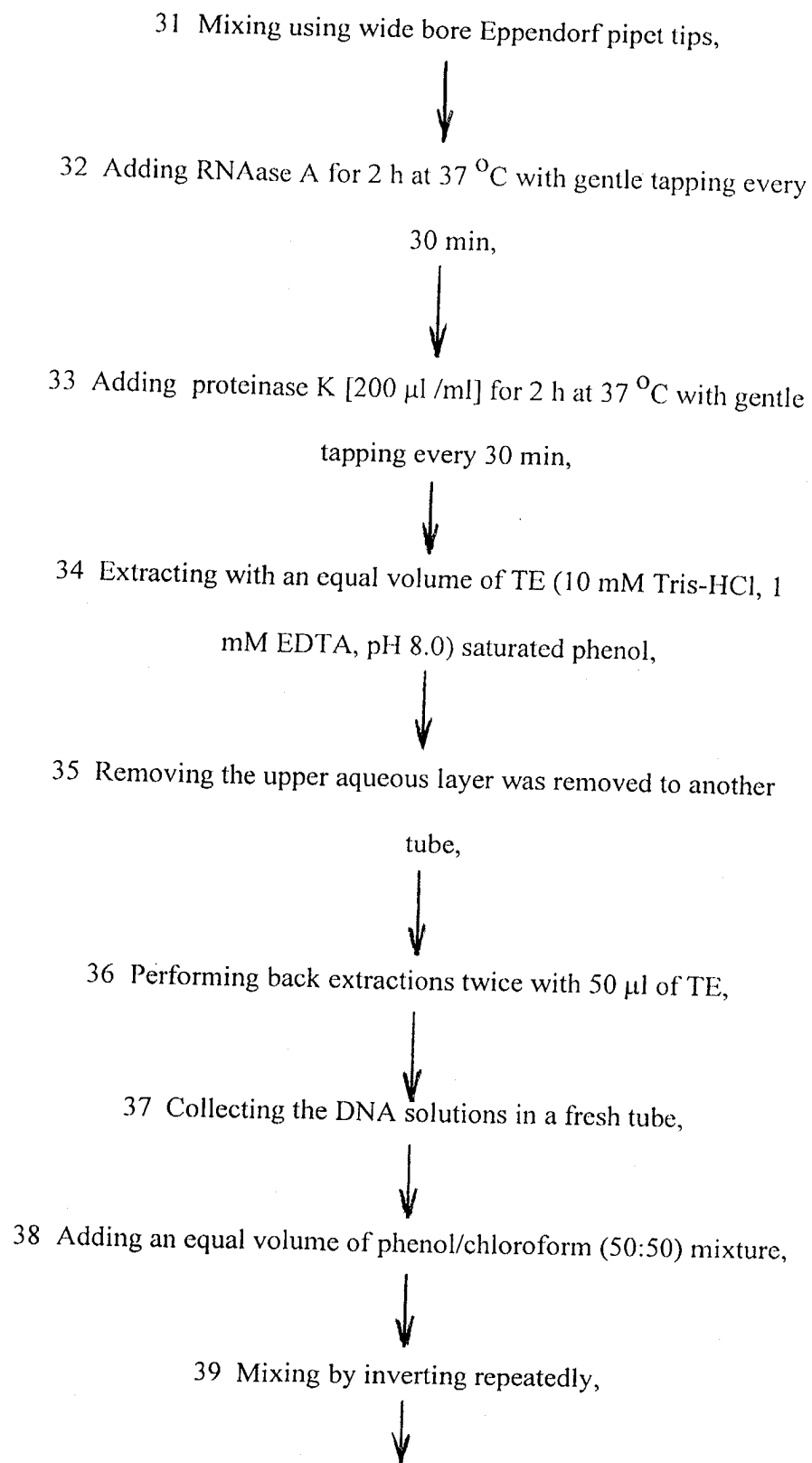


Fig. 4 F

APPROVED	G. G. FIG.	
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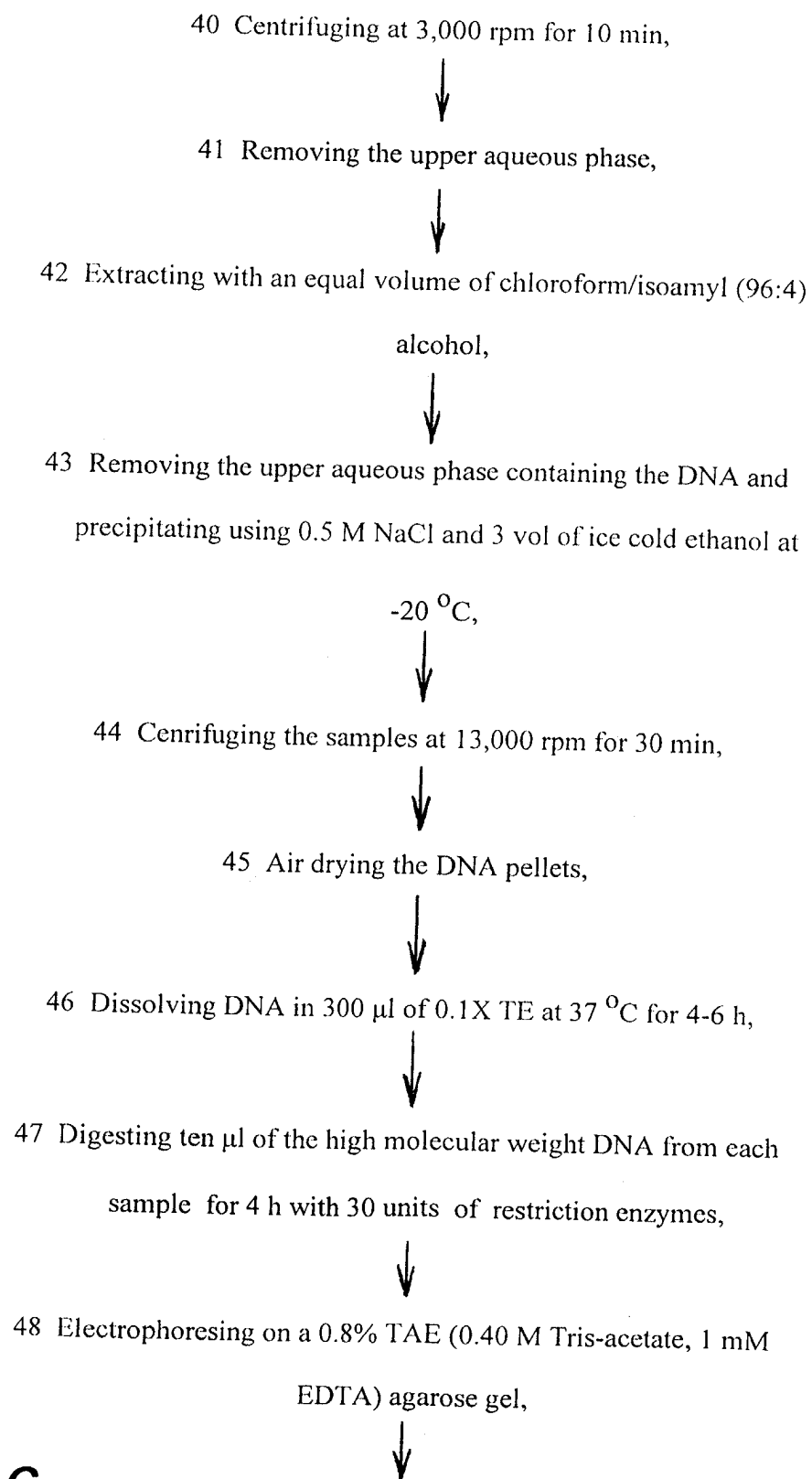


Fig. 4 G

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49 Staining the DNA gel with ethidium bromide,



50 Photographing with UV light,



51 Radiolabeling of the sequence specific oligonucleotides
comprising the steps of,



52 Synthesizing single stranded oligonucleotides on a DNA
synthesizer and annealing with the complimentary strand by
combining 4 μ g of both strands in a tube with total volume of
30 μ l of annealing buffer (5 mM NaCl, 10 mM Tris-HCl and 0.
2 mM EDTA),



53 boiling the tubes for 5 min and slowly cooling to room
temperature for 6 h,



54 Heating the tubes to 55 $^{\circ}$ C for 5 min and then cooling on ice
for 10 min,



55 Quantitating 4 μ l aliquot of the annealed oligos at 260 λ and
storing the remainder at -20 $^{\circ}$ C until radiolabeled,



Fig. 4 H

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APPROVED	D.E. FIG.	
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56 Preparing probes by radiolabeling 200 ng of annealed oligonucleotide in 15 μ l of total volume containing 50 μ Ci of [γ - 32 P]ATP (6,000 Ci/mmol), 20 Units of T4 polynucleotide kinase, and 1.5 μ l 10X T4 polynucleotide kinase buffer for 1 h

at 37 $^{\circ}$ C,



57 Filling in the 5' over-hang ends with 5 Units of Klenow with 3.0 μ l of 10X Klenow buffer and 0.15 mM each of dATP, dCTP, dGTP, and dTTP for 40 min at 37 $^{\circ}$ C in a reaction

volume of 30 μ l.



58 Increasing the volume to 1 ml with sterile TE with 200 mM NaCl, pH 8.0, and the labeled oligonucleotides were purified on a NACS Prepac column, to separate the unincorporated

nucleotides,



59 Precipitating the labeled, purified oligonucleotides overnight

with 3 vol absolute ethanol at -20 $^{\circ}$ C,



Fig. 4 I

APPROVED	D.S. FIG.	
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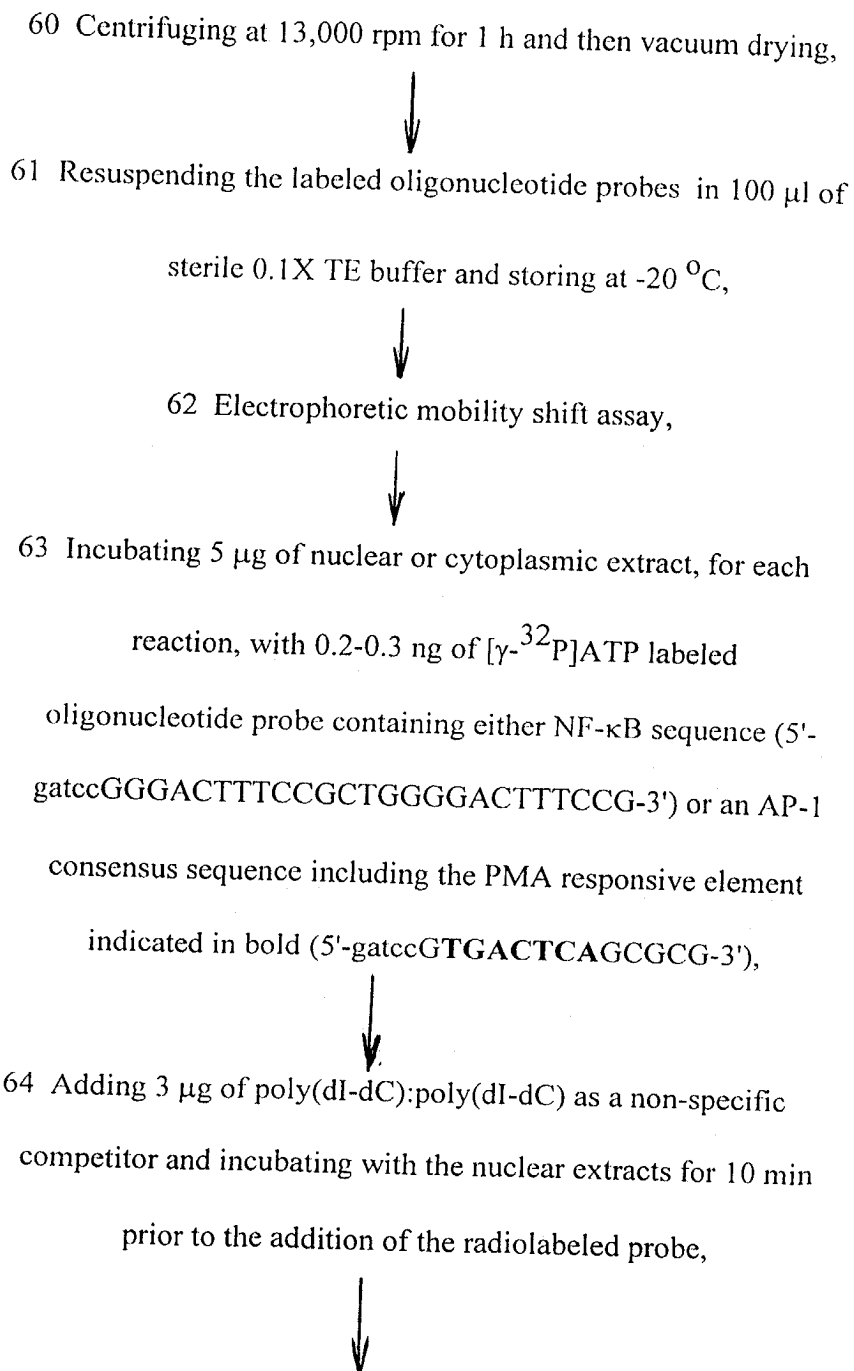


Fig. 4 J

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APPROVED	O.G. FIG.	
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65 Adding antibodies against p65, p50, c-*Fos* or c-*Jun* to the respective binding reactions for supershift assays and incubating at room temperature for 1.5 h, prior to probing with [γ -³²P]ATP labeled oligonucleotide for an additional 25 min at

room temperature,



66 Separating the bound complexes on either a 5% ,for supershift assays, or 6% ,for analytical purposes, acrylamide/bis (30:1 ratio) native gel as required and running at 200v for 3.5 h with 0.25X TBE (0.02 M Tris-borate, 0.5 mM EDTA) as running

buffer at room temperature,



67 Vacuum drying the gel with heat at 80 °C and exposing them to Kodak X-OMAT film for 3-12 hours,



68 Analyzing the bound and free DNA protein complexes.

Fig. 4 K